RISK ASSESSMENT – TASK BASED
IGEM 2016

Location:	Building Number:	Date:	Assessed By:	Health & Safety Representative:
Room W301, Medical Building	181	February 2016	Amber Willems Jones	Vincé Kalangi

Description of Activity: **4.2 PCR** **SWP 4.2**	
Is there past experience with the Activity that may assist in the risk assessment? Incidents & Near-hits, Incident Investigations, Workplace Inspections, Training, Standards, Legislation & Codes, Uni Guidance Material, Existing Controls, Industry Standards.	NO

1.TASK	2.HAZARD	3.Estimated RAW RISK SCORE C x E x L	4.CONTROLS	5. Residual Risk Score RISK SCORE C	E	L	C x E x L	6. Residual Risk
Buffer prep for PCR	skin contact with MgCl2 Causes eye and skin irritation	5x3x1	Personal Protective Equipment ; training	5	3	0.1	1.5	Low risk
	TOTAL	15		TOTAL			1.5	Low risk

Name & Signature of Laboratory Head/Supervisor or Delegate	Amber Willems Jones	Date	
Name & Signature of Person Performing Activity or Task		Date	

Number and Title	PRG 4.2 PCR
Name of Laboratory/Department	The University of Melbourne IGEM Team Laboratory/Department of Biochemistry
Author, Date Prepared & Date of Review	Author: Ella Bocquet-Gaylard Date: 22/2/2016 Updated : February 2016, Review by: February 2018
Introduction	The **polymerase chain reaction** (**PCR**) is a biochemical technology in molecular biology to amplify a single or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.
Principles / Scope	Amplify DNA sequence
Risk Management	***Risk assessments have been prepared and are available attached to the SWP*. Raw Risk:Residual Risk:** Low Risk
Safety Management	**Hazards: Eye and skin irritation** **Risk Controls: Low Risk**
Licences / Permits	N/A
Training / Competency	All team members must be inducted to the use of any equipment used.
Equipment	Thermal cycler equipment Power supply
Protocol	***1Preparation of Primers*** Materials sterile distilled water Primers supplied *Note: The amount of primer to use in any given PCR reaction can be considered as a variable.* Typically usually use 25 pmol/µl concentration of primers in each reaction:
Step 1	Calculate the amount of dH20 required to make the concentration of primers to 25 nmol/ µl ie $\frac{36.4 \text{ nmol}}{x} = \frac{25 \text{ nmol}}{100 \text{ ml}}$ => x = 143 µl
Step 2	Dilute this stock of primer 1/10 to give a working primer stock of 250 pmol/ µl and use 1 µl of this primer is subsequent PCR reactions. *Note, ensure to write the concentration of primers and the amount of dH20 added to the primers for the next user.

2 PCR reaction

Materials
sterile distilled water
Primers
10 X reaction buffer (eg Invitrogen DNA polymerase PCR buffer)
25 mM MgCl
Taq polymerase

It is recommended that many reactions be prepared at once, to ensure an appropriate amount of DNA product is yielded to continue with the cloning process.

Make a 'master mix' that will be enough for 5 reactions, but one of these is going to be a negative control. It is extremely important to ALWAYS do a negative control to ensure that there has not been a plasmid contamination.

General notes. Always add the dH2O first. Try and space the addition of the primers i.e. add one primer and then the reaction buffer and then the other primer, just in case the primers associate with themselves.
Set up the following 5x MM reaction

Reagent	**volume to be added**
Reaction Buffer 10X	25 µl
25 mM dNTP mix	2.5 µl
25 mM MgCl2	3.0 µl
Forward primer	5.0 µl
Reverse primer	5.0 µl
dH20	203.75 µl
Taq	0.75 µl

** Take out 50 µl for the 0 DNA control

Step1

Add Template DNA (100ng) 4u L

Step 2

Aliquot out into 4x 50 µl reactions.

Step 3

3 PCR cycle

Consider the Tm of your primers
Ie if you had two primers with Tm 67 and 65C you would set up the following protocol

Cycle 1 (x 2 cycles)

94 C	0.30 sec	(denature)
67 C	0.45 sec	(annealing)
72 C	1.00 min	(extension)

Cycle 2 (x25 cycles)

94 C	0.30 sec

	65 C 0.45 sec 72 C 1.00 min 72 10 min 4 hold If there were more than just a few degrees between the Tm of your primers its best to do a step wise reduction in the annealing step (two cycles for each step until the final); ie, if your Tms were 70 and 65 do **Cycle 1 (**x 2 cycles) 94 C 0.30 sec (denature) **70** C 0.45 sec (annealing) 72 C 1.00 min (extension) **Cycle 2** (x2 cycles) 94 C 0.30 sec **67** C 0.45 sec 72 C 1.00 min **Cycle 2** (x25 cycles) 94 C 0.30 sec **65** C 0.45 sec 72 C 1.00 min 72 C 10 min 4 C hold *Note: Annealing temperature will change depending on the T_m of the primers which can be calculated as follows: 4 x (G + C) + 2 x (A + T). Ideally the T_m of the primers should be within 5 ºC of one another. Maintain at 4°C after cycling. Withdraw a sample from each of the reaction mixtures and analyse by agarose gel electrophoresis, including DNA markers above and below 1kb.
Controls / Calibration	N/A
Waste Disposal	biohazard bin
Emergency Procedures	First aid measures Eye contact: Immediately flush eyes with plenty of water for at least 20 minutes and get medical attention. Skin contact: In case of contact, immediately flush skin with plentyof water for at least 20 minutes. Inhalation: Move exposed person to fresh air. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. Get medical attention.

	Ingestion: Wash out mouth with water. Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Call medical doctor or poison control centre immediately.
References	
Authorised By	Amber Willems Jones